Date.	Nitrogen in diet.	Protein in diet.	Nitrogen in urine.	Equivalent in urea.	Water ingested.	Water ingested as milk.	Urine passed.	P_2O_5 excreted.	Weight.
March 15	11.330	70.812	10.395	22.275	1500 c.c.	609.87 c.c.	1650 c.c.	1.749	64.8 k. (142¾ lbs)
" 16	8.755	54.718	9.2046	19.7242	907 "	682.95 "	1105 "	1.2818	64.8 k. (142¾ lbs)
" 17	10.569	66.056	9.5711	20.5095	985 "	677.73 "	1210 "	1.7303	64.8 k. (142¾ lbs)
" 18	10.966	68.537	12.0204	25.7580	1155 "	783.00 "	1080 "	1.674	64.5 k. (142¼ lbs)
" 19	8.264	50.650	10.6543	22.8307	998 "	468.06 "	1095 "	1.7191	65.3 k. (144 lbs)
" 20	7.978	49.862	7.476	16.02	1071 "	875.84 "	600 "	1.158	64.8 k. (142¾ lbs)
" 21	7.534	47.087	9.4136	20.172	1299 "	486.33 "	820 "	1.5252	64.9 k. (143 lbs)
Total	65.396	407.722	68.735	147.2694	7915 "	4083.78 "	7560 "		

REFERENCES.

1. W. O. Atwater and Charles D. Woods. The Chemical Composition of American Food Materials, United States Department of Agriculture, Bulletin, No. 28, 1896.

2. W. O. Atwater and Charles D. Woods. Dietary Studies in New York City in 1895 and 1896, Ibid., No. 46, 1898.

3. W. D. Halliburton. Text-book of Chemical Physiology and Pathology, 1891.

4. A. E. Parkes. Proceedings of the Royal Society, London, 1867.

5. Gruber. Zeit. f. Biol., Bd. xiii. p. 407.

6. Straub. Ibid., Bd. xxxviii. p. 537.

7. N. S. Davis, Jr. Volume on Dietotherapy and Food in Health, System of Physiologic Therapeutics, edited by S. Solis-Cohen. p. 32

8. Sivén. Skand. Arch., Bd. x. p. 91.

9. Elbert W. Rockwood, American Journal of Physiology, vol. xi. No. 4.

ON THE DETECTION OF MINUTE TRACES OF SUGAR IN THE URINE.

By Ernest L. McEwen, M.S., M.D.,

ASSISTANT INSTRUCTOR IN DERMATOLOGY, RUSH MEDICAL COLLEGE, CHICAGO.

Practically all authorities admit that traces of sugar may occur in the urine of perfectly normal individuals, especially if the ingestion of carbodydrates has been excessive. Although such traces do not concern the average practitioner, since the condition giving rise to their presence is scarcely pathological, it is of interest scientifically to consider how these minute quantities of sugar may be detected, and notably so since methods of such detection are not discussed by the various authorities on urinalysis. In fact, that the test hereinafter considered actually demonstrates the presence of minute quantities of sugar in urine is not generally accepted, and it is the purpose of this paper to show why such delicacy may properly be attributed to it.

In 1884 Emil Fischer[1] first called attention to the fact that phenylhydrazin, in combination with certain carbohydrates, formed definite and characteristic crystalline compounds. His work upon the subject was scientific and quite exhaustive, though he barely more than suggested a practical application. A few years later von Jaksch and Grocco gave the test its introduction as a clinical procedure, and to the former is due largely the high esteem in which it has been held as a means of detecting sugar in glycosuria. It has since been studied by a number of investigators, notably among the Germans, but conclusions thus far have not been entirely uniform.

Briefly speaking the test consists in treating a portion of suspected urine in a test-tube with phenylhydrazin hydrochlorate and sodium acetate, heating, cooling, and allowing the tube to stand for some time, then examining the resulting sediment microscopically. As to the proportion of ingredients used nearly all authorities are agreed, recommending that 10 c.c. of urine be mixed with 1 gm. of phenylhydrazin and 2 gms. of sodium acetate. Regarding the manner and duration of boiling, opinions differ. Fischer used a water bath, boiling the tube ten to fifteen minutes; von Jaksch proceeded similarly, but boiled the tube longer. Hirschl[2] claimed that by boiling in a water bath one hour an absolute differentiation between glucose and glycuronic acid could be made; while Williamson[3] considered that prolonged heating introduced certain fallacies and advised that the test-tube be boiled for two minutes only, directly over the lamp. All are quite agreed that prolonged standing serves to bring out the crystals more perfectly. Disregarding these differences of opinion as to details, we may say that the accepted method of applying the test to-day is as follows: To 10 c.c. of urine in a test-tube 1 gm. of phenylhydrazin hydrochlorate and 2 gms. of sodium acetate are added; the tube is heated in a boiling water bath for one hour; it is then allowed to stand twenty-four hours, when a microscopic examination of the sediment at the bottom of the tube is made; if sugar has been present in the urine, certain yellow crystalline forms are to be seen.

It is at this point that confusion and doubt have arisen, for the crystals produced are not of one uniform type. A survey of the literature discloses that the crystals constantly mentioned as present are divisible into two general types. One of these is universally acknowledged to be the genuine phenylglucosazone crystal, and is described as being composed of delicate, sulphur-yellow needles, beautifully arranged in sheaves, half-sheaves, rosettes, and sprays. Its appearance is proof positive of the presence of sugar. The other type is that of a much smaller crystal of the same color, a conception of its form and appearance being quite accurately conveyed by the word "thornapple," meaning thereby the seedpod of *Datura stramonium.* It is seen in short, boat-shaped staves with few or many centrally disposed radiating spiculæ, and has been likened, save for its color, to the crystal of ammonium urate, neutral calcium phosphate, and hippuric acid. It has been observed repeatedly by different investigators in diabetic urine, in artificially saccharine urine, and in normal urine, after the application of the phenylhydrazin test. Approximately three views are held as to the significance of this crystal: first, that it is to be disregarded totally as having no import;[5] second, that it indicates the presence of glycuronic acid or some unknown constituent of urine;[6] third, that it signifies the certain presence of sugar.

Considering these views seriatim it may be said, with respect to the first, that the crystal has some import, since in many specimens examined it is not found. Its presence or absence therefore indicates the presence or absence of some urinary constituent.

The second view is more vital. Glycuronic acid forms with phenylhydrazin yellow crystals which are scarcely to be distinguished microscopically from those of glucosazone. Thierfelder[6] investigated this combination extensively. Later Hirschl[2] described the glycuronic acid phenylhydrazin crystals as follows: "Out of a central mass many needle-like spiculæ project, to become merged immediately into other and similar masses; the centre of the radially arranged needles appears as an irregular mass out of which the needles seem to be growing." Jolles[8] accepts this description, but adds that the glycuronic acid crystals are shorter and thicker than the sugar crystals, and are never so regularly arranged; their disposition is generally in rosettes, approaching in appearance the thornapple type; he emphasizes the statement that the glucosazone crystals are long and slender as compared with the acid crystals.

The experiments which the writer has made confirm in the main the accuracy of Hirschl's description of the glycuronic acid crystals. In the majority of tests made aqueous solutions of glycuronic acid treated with phenylhydrazin (in the same manner as urine) gave a greenish-brown viscus sediment, which, under the microscope, was seen to be composed of many rounded masses, with minute needle-like crystals projecting from their edges. In a few instances the crystals were obtained free of the central mass and appeared as very fine needles, arranged in a radiating manner; by pressure upon the cover-slip the rosettes could be broken up into segments, each exactly resembling a half-sheath glucosazone crystal. Jolles' observation as to the thickness of the crystals was not confirmed; on the contrary, their extreme tenuity was a striking feature.

A point which must be regarded as extremely important is that repeated attempts to remove the glycuronic acid crystals by previous fermentation with yeast were uniformly unsuccessful. Solutions of glycuronic acid in water and in urine were subjected to this procedure, with the invariable result that the acid was not removed, since subsequent treatment with phenylhydrazin always produced the characteristic crystals.

Respecting the third view, that the thornapple crystal indicates the presence of sugar, the results of experimentation given below are of interest, and seem to warrant confirmatory deductions. The writer subjected 100 different urines from apparently healthy individuals to the phenylhydrazin test with careful reference to technique; in 94 a precipitate containing yellow crystals in varying amounts was found. The 100 samples thus tested were all negative to the copper test with one exception; this reduced Haines' solution vigorously and the fermentation test showed 3¾ per cent. of sugar; the crystals produced with phenylhydrazin were absolutely typical of sugar. In the 93 other cases the type of crystal was approximately the thornapple, appearing in completed form or in component parts, the finished crystal apparently being produced by the cleavage and spiculation of a fundamental boat-shaped crystal, with subsequent aggregation of these units into masses exactly resembling an echinoderm or "thornapple," as mentioned above.

This observation corroborates the findings of several investigators. Pavy[10] and Allen[11] secured crystals readily from healthy urine. Kisterman[6] in 100 specimens of normal urine found crystals of a thornapple type in every case; these as far as form was concerned could not be distinguished from the crystals of glucosazone obtained from weak aqueous solutions of sugar. Roos[12] examined 16 normal urines, finding crystals in each "characteristic of sugar." Frank[7] in all urines examined found "platelets" and "thornapple forms resembling rosettes" among the amorphous material. Moritz[13] found crystals in nearly every case, and Williamson[3] obtained a like result. Geyer[14] believed that a crystalline deposit could be obtained from all urines. Schilder,[15] by direct methods, proved that the constantly present crystals which he observed indicated sugar. MacDonald[16] found crystals in over one-half of all cases. Further enumeration of similar observations is scarcely necessary to establish fairly that a yellow crystalline precipitate is formed in the majority of all specimens of urine examined by the phenylhydrazin test.

Having observed the almost constant appearance of the thornapple crystals, an effort was made to discover their nature. By adding a very low percentage of grape-sugar to urine which normally gave the crystals, it was found that their number was greatly augmented; with increasing percentages a point was reached—about 0.01 per cent.—where the typical sugar crystal appeared. Further, it was found that by first subjecting the urine to thorough alcoholic fermentation the sediment was invariably amorphous. This was abundantly proven by repeated tests. Williamson[3] obtained precisely similar results; he regarded prolonged fermentation as essential to remove all traces of crystals on the ground that minute quantities of sugar are removed slowly with yeast. On the other hand, Geyer[14] obtained crystals after fermentation. It is probable that prolonged fermentation is advantageous, since it is then more apt to be complete; in the experience of the writer twenty-four to thirty hours sufficed.

In consideration of all the facts set forth in the above, the following recapitulation may be made:

1. Two forms of crystals may appear in urine after treatment with phenylhydrazin: one composed of slender needles arranged in the form of sheaves and half-sheaves is typical of sugar; the other, much smaller, composed of radiating spiculæ, aggregated into spinous masses, has been variously interpreted.
2. Glycuronic acid in urine produces a crystal very similar to the true sugar crystal. Its component filaments are very slender, and are never short and thick, as may be the case in the thornapple crystal.
3. Glycuronic acid crystals cannot be removed by previous fermentation with yeast.
4. The thornapple crystal appears in a very large proportion of all urines examined, and increases in number as low percentages of sugar are added to urine normally showing them. A point is ultimately reached in this addition where typical crystals appear.
5. Both the typical crystal and the thornapple crystal can be removed by previous fermentation with yeast.
6. It may therefore be said, in conclusion, that the small "thornapple" crystal indicates the presence of sugar, that practically all urines contain traces of sugar, and that the phenylhydrazin test will detect its presence.

BIBLIOGRAPHY.

1. Fischer. Bericht der Deutsch. Chem. Gesellsch. zu Berlin, xvii., 579.
2. Hirschl. Zeitsch. f. physiol. Chem., xiv., 377.
3. Williamson. Manchester Med. Chronicle, 1895, 333.
4. Bond. Med. News, 1887, ii., 146.
5. Beane. N. Y. Med. Journ., 1893.
6. Kisterman. Deutsch. Arch. f. klin. Med., Leipzig, 1892, i., 423.
7. Frank. Berliner klin. Wochenschr., 1893, xxx., 255.
8. Jolles. Centralbl. f. innere Med., 1894, 1047.
9. Thierfelder. Zeitschr. f. physiol. Chem., 1887, ii., 388.
10. Pavy. London Lancet, July 14, 1894.
11. Allen. Ibid., July 28, 1894.
12. Roos. Zeitschr. f. physiol. Chem., 1891, xv., 523.
13. Moritz. Münchener. med. Wochenschr., 1891, i. and ii.
14. Geyer. Am. Journ. Med. Sci., February, 1890.
15. Schilder. Wiener med. Blätter, 1896, 13.
16. MacDonald. London Lancet, 1896, ii., 814.